Case Report

Primary Aldosteronism Caused by a Unilateral Adrenal Adenoma Accompanied by Autonomous Cortisol Secretion

Jiro SUZUKI[1)], Fumio OTSUKA[1)], Kenichi INAGAKI[1)], Hiroyuki OTANI[1)], Tomoko MIYOSHI[1)], Tomohiro TERASAKA[1)], Toshio OGURA[1)], Masako OMORI[2)], Yasutomo NASU[3)], and Hirofumi MAKINO[1)]

A 35-year-old Japanese woman was referred for further examination of persistent hypertension with hypokalemia. Her serum aldosterone levels were high and her plasma renin activity markedly suppressed. Radiological examinations revealed the presence of a 3-cm diameter left adrenal tumor. ^{131}I-adosterol was specifically accumulated in the left adrenal tumor, whereas the accumulation in the right adrenal was completely suppressed. Low-dose dexamethasone failed to suppress cortisol secretion although the serum cortisol levels were within the normal range. Urinary excretion of 17-hydroxycorticosteroids but not 17-ketosteroids was increased. Levels of plasma adrenocorticotropin (ACTH) and serum dehydroepiandrosterone sulfate (DHEAS) were decreased. Upon diagnosis of left aldosteronoma with autonomous secretion of cortisol, left adrenalectomy was performed by laparoscopy. In the resected adenoma tissues, clear cells expressed P450c17 protein and the ratio of CYP17/CYP11B2 mRNA evaluated by quantitative real-time polymerase chain reaction (PCR) was apparently higher than that of typical aldosteronomas. Based on the corticotropin-releasing hormone (CRH) loading tests, the contra-lateral adrenal functions were restored 3 months after surgery. These results indicate that evaluation for autonomy of cortisol secretion and contralateral adrenal function is clinically important to avoid the risk of adrenal failure after surgery for primary aldosteronism. (*Hypertens Res* 2007; 30: 367–373)

***Key Words*: CYP11B2, CYP17, P450c17, preclinical Cushing's syndrome, primary aldosteronism**

Introduction

Primary aldosteronism results from overproduction of mineralocorticoid hormone from the zona glomerulosa of the adrenal cortex, and is one of the major causes of secondary hypertension (*1*). The etiology is attributable to unilateral lesions, including aldosterone-producing adrenocortical adenoma/carcinoma and unilateral hyperplasia (*2*), or bilateral adrenal hyperplasia (also termed idiopathic hyperaldosteronism), although it can be difficult to distinguish among these entities in the early clinical stage (*3*). In rare cases, glucocorticoid-remediable hyperaldosteronism may be diagnosed in young adults with a family history of hypertension with hypokalemia (*4*). Although primary aldosteronism was originally defined as Conn's syndrome, a condition that is not associated with cortisol oversecretion, unusual conditions showing concurrent secretion of aldosterone and cortisol from a single adrenal tumor have been described (*5*–*7*). Recognition of the presence of cortisol excess is clinically important to avoid adrenal failure after surgery for primary aldosteronism due to unilateral lesions.

From the [1)]Department of Medicine and Clinical Science, [2)]Department of Pathology, and [3)]Department of Urology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan.

Address for Reprints: Fumio Otsuka, M.D., Ph.D., Department of Medicine and Clinical Science, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, 2–5–1 Shikata-cho, Okayama 700–8558, Japan. E-mail: fumiotsu@md.okayama-u.ac.jp

Received July 28, 2006; Accepted in revised form December 4, 2006.

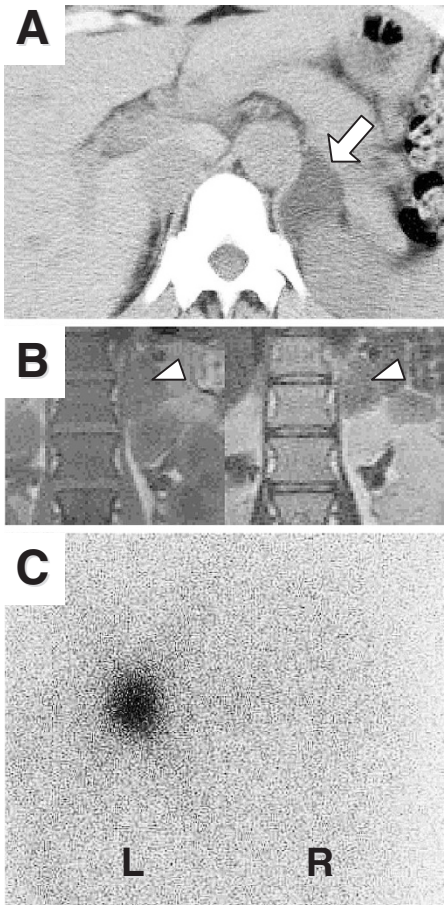

Fig. 1. *Radiological findings. A: Plain adrenal CT. An arrow indicates the adrenal tumor. B: Adrenal MRI in* T_1 *(left)- and* T_2 *(right)-weighted images. Arrowheads indicate the adrenal tumor. C: Adrenal cortical scintigraphy with* ^{131}*I-adosterol. Note the significantly high uptake of the left adrenal tumor and complete suppression in the right adrenal region.*

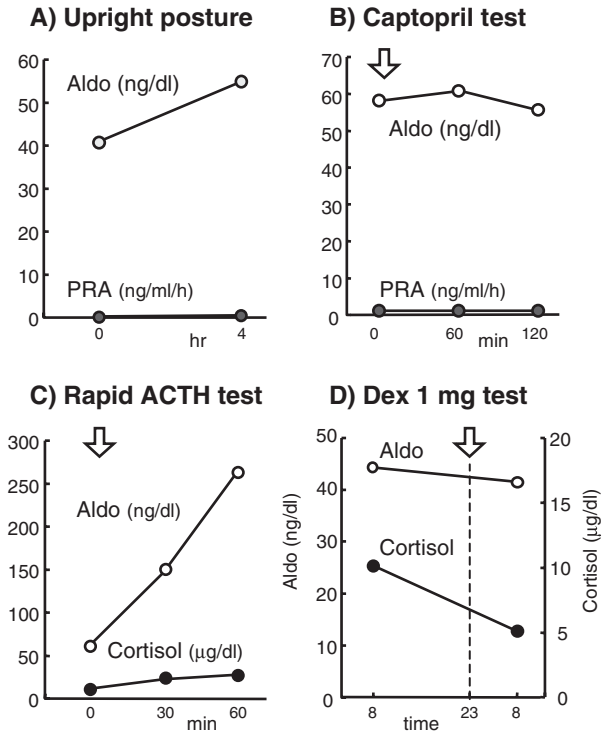

Fig. 2. *Preoperative endocrine tests. A: Four-hour upright posture test. B: Captopril (25 mg, orally) test. C: ACTH (0.25 mg, i.v.) loading test. D: Dexamethasone (Dex; 1 mg, orally) suppression test. Aldo, aldosterone; PRA, plasma renin activity.*

Here we report a patient with primary hyperaldosteronism due to a single adrenocortical adenoma that concomitantly displayed autonomous secretion of cortisol, *i.e.*, hyperaldosteronism with preclinical Cushing's state. Molecular approaches have revealed relatively abundant mRNA expression of CYP17 compared with CYP11B2 in the resected adrenal tumor. We also demonstrated the time-course of the recovery of contra-lateral adrenal suppression after adrenalectomy.

Case Presentation

A 35-year-old Japanese woman who had a 3-year history of hypertension was referred to our hospital for evaluation of hypokalemia. Her blood pressure was 159/112 mmHg and her pulse rate was regularly 79 bpm. She was of normal stature, with a height of 159.2 cm, weight of 54.8 kg, and body mass index of 21.6 kg/m^2. She had experienced no unusual increase in body weight or menstrual abnormality. No typical features of Cushing's syndrome, such as moon face, truncal obesity, buffalo hump, muscle weakness, hirsutism, or striae cuitis, pigmentation or atrophy of the skin were observed. Laboratory data showed persistent hypokalemia (2.6 mEq/l) and increased urine potassium secretion (2.35 g/day). Eosinophil counts were moderately decreased to 0.8–1.8% (51–75/μl). Liver and renal functions and glucose tolerance were found to be normal.

CT of the abdomen demonstrated a 3-cm diameter adrenal tumor in the left adrenal region (Fig. 1A). The adrenal tumor exhibited very low CT density (−2 HU) and was marginally enhanced with early washout, and also showed homogenously low intensities by T_1- and T_2-weighted magnetic resonance images, compatible with a benign adrenocortical adenoma replete with adipose tissue (Fig. 1B). The right adrenal showed no specific findings by thin-slice CT scans. Adrenal cortical scintigraphy with ^{131}I-adosterol exhibited a unilateral uptake of the left adrenal region with complete suppression of the right adrenal gland (Fig. 1C).

Endocrine examinations taken with the patient in a recumbent position revealed that the serum aldosterone concentra-

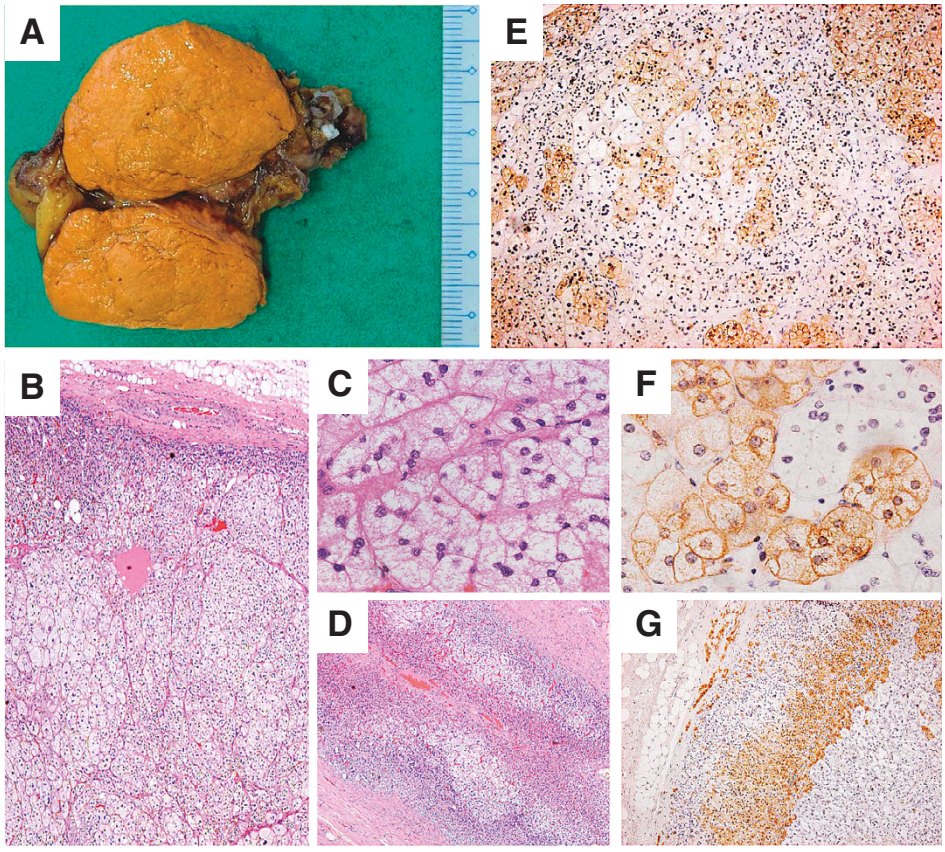

Fig. 3. *Pathological findings of the resected adrenal tissues. A: Macroscopic findings. B–D: Microscopic findings by hematoxylin-eosin staining. The adrenal adenoma tissues appeared to be composed mainly of large clear cells at both lower (B) and higher (C) magnification. Paradoxical hyperplasia was shown in the zona glomerulosa in the adjacent adrenal tissues (D) while atrophic changes in the normal adrenal cortex were not observed. E–G: Immunohistochemical study by anti-P450c17 antibody. Clear cells in the adrenal adenoma were P450c17-positive at both lower (E) and higher (F) magnification. The zona fasciculata in the adjacent adrenal clearly expressed P450c17 protein (G).*

tion was markedly elevated (673 to 300 pg/ml; normal: 30–159) and plasma renin activity (PRA) was consistently suppressed (0.2 ng/ml/h; normal: 0.2–2.7) from morning till night. Urinary aldosterone excretion was also increased to 68.3 μg/day (normal: 0–7.5). Both upright-posture test (for 4 h) and captopril (25 mg, orally) administration, which normally evoke renin release, were totally ineffective at increasing PRA (Fig. 2A and B). Adrenocorticotropin (ACTH) stimulation (0.25 mg, i.v.) induced a remarkable rise in aldosterone to more than 4-fold the basal level (Fig. 2C). Serum cortisol levels at 8:00 AM were within the normal range (~10 μg/dl). However, cortisol secretion lacked circadian changes and low-dose dexamethasone (1 mg, orally) failed to suppress serum cortisol secretion to below 3 μg/dl (*8*) (Fig. 2D). Plasma ACTH levels were lowered (~5 pg/ml) without diurnal fluctuation and serum dehydroepiandrosterone sulfate (DHEAS) levels were decreased (290 ng/ml; normal: 500–1,710). Urinary excretion of 17-hydroxysteroids was marginally high (8.5 mg/day; normal: 2.2–7.3) while that of 17-ketosteroids was within the normal limits (5.3 mg/day; normal: 2.4–11).

Upon diagnosis of the tumor as a left adrenocortical functioning tumor secreting predominantly aldosterone, left adrenalectomy was performed by laparoscopic surgery. The cut surface of the tumor was golden-yellow in color (Fig. 3A), which was leading to a pathological diagnosis of adrenocortical adenoma mainly composed of large clear cells (Fig. 3B and C). Moderate hyperplasia was observed in the zona glomerulosa of the non-neoplastic adrenocortical tissues, while no atrophic lesion was found in the zona fasciculata and reticulata of the adjacent adrenal (Fig. 3D). Immunohistochemical study with anti-P450c17 antibody revealed spotty expression of P450c17 protein in clear cells composing the adrenocortical adenoma (Fig. 3E and F) as well as the zona fasciculata of the adjacent adrenocortical tissues (Fig. 3G), indicating that the aldosterone adenoma had the ability to produce cortisol.

The patient received oral hydrocortisone supplementation (10 to 5 mg/day) for 2 months post-surgically. Preoperatively,

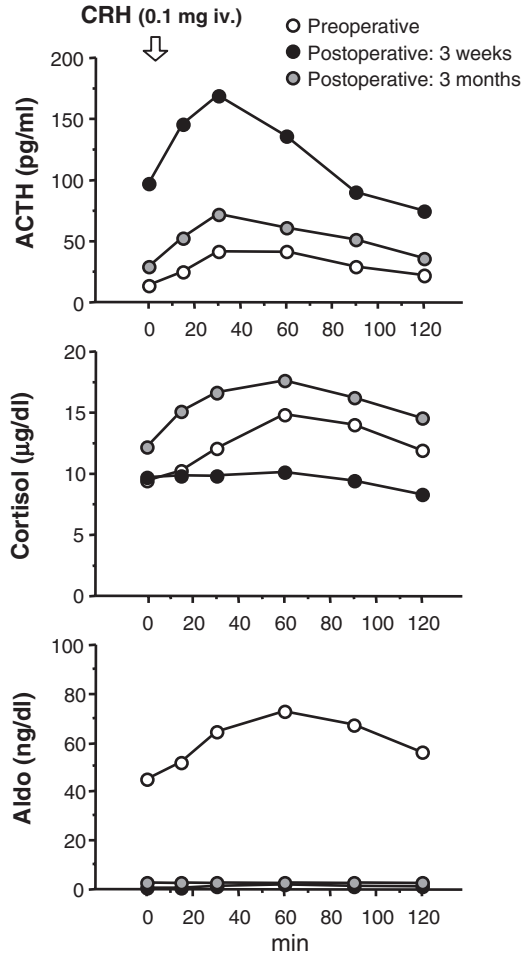

Fig. 4. *CRH loading tests. Time-course changes in ACTH, cortisol and aldosterone (Aldo) in response to CRH (0.1 mg, i.v.) were evaluated before (open circles), 3 weeks after (closed circles) and 3 months after (gray circles) the adrenal surgery.*

ACTH had shown a weak response to a corticotropin-releasing hormone (CRH; 0.1 mg, i.v.) loading test (Fig. 4). At 3 weeks after surgery, with oral hydrocortisone having been administered throughout this period, CRH stimulation evoked no response of cortisol secretion from the remnant right adrenal despite hyperresponsiveness of ACTH (Fig. 4), while the aldosterone excess completely disappeared. Because symptoms of adrenal insufficiency, such as hypotension, hyponatremia, hypoglycemia and general fatigue, had not been observed without taking hydrocortisone since 2 months after surgery, cortisol replacement was discontinued. At 3 months after surgery, the responsiveness of pituitary corticotropes and the remaining adrenal response to CRH were found to be normal (Fig. 4).

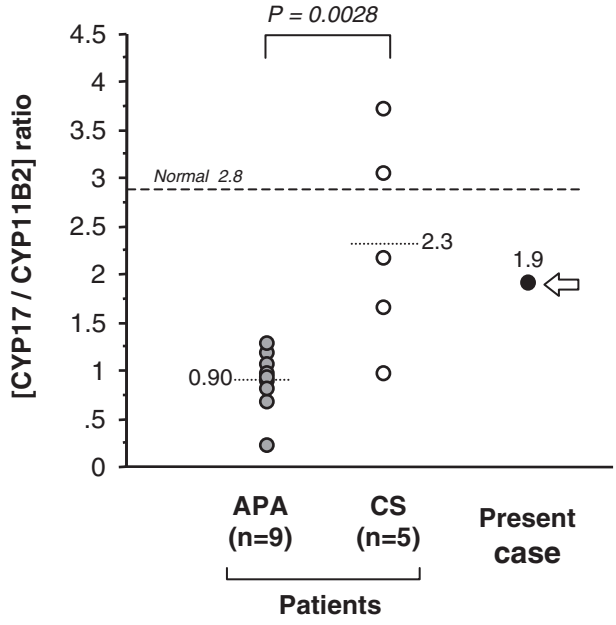

Fig. 5. *Ratios of CYP17/CYP11B2 mRNA in adrenal adenomas. Steady-state levels of mRNAs encoding CYP17 and CYP11B2 were examined by quantitative real-time PCR analysis and the results were standardized by RPL19 levels. The results are shown as the ratio of CYP17 to CYP11B2 (mean±SEM from 3 repeated experiments). APA, aldosterone-producing adenomas; CS, Cushing's adenomas. An arrow indicates the data from our present case. A dotted line shows the data from normal whole adrenal RNA. Student's* t-*test was used to compare APA with CS. Values of* $p < 0.05$ *were considered to indicate statistical significance.*

Expression Levels of CYP11B2 and CYP17 mRNA in Adrenal Adenomas

To determine the characteristics of steroidogenesis in adrenal tumors, we examined mRNA expression levels of CYP17 and CYP11B2 encoding P450c17 and P450aldo, respectively. Human adrenal RNA samples were collected from adrenocortical tumor tissues from patients who had been diagnosed, using clinical and pathological criteria, with aldosterone adenoma (9 patients: 7 males and 2 females; aged 49.3±3 years) or cortisol adenoma (5 patients: 1 male and 4 females; aged 41.2±6 years). Normal whole-adrenal RNA (Stratagene, San Diego, USA) was used as a control. Informed consent and written permission from each individual for the experimental use of the tissues were obtained in advance of the surgery. Total cellular RNA was extracted using TRIzol® (Invitrogen, Carlsbad, USA) and 1 μg of RNA was subjected to reverse transcription (RT) reaction using a First-Strand cDNA Synthesis System® (Invitrogen). The GenBank accession numbers of the primers were as follows: CYP17 (M14564: 661–681 and 880–900), CYP11B2 (NM_000498: 704–723 and

Table 1. Literature Review of Primary Aldosteronism with Autonomous Cortisol Secretion

Age/Sex	Typical Cushing's symptoms	Systemic BP (mmHg)	Serum K (mEq/l)	Tumor size (cm)	Aldo (ng/dl)		PRA (ng/ml/h)	Cortisol (μg/dl)		
					Basal	ACTH response		Basal	Circadian rhythm	1 mg Dex suppress
34/F	(−)	220/120	2.6	Lt 1.3	107.2		0.15	17.1	(−)	(−)
40/F	(−)	226/140	2.4	Lt 3	75.2	(+)	0.1	11.1		(−)
73/F	(−)	186/98	2.6	Rt 3	55.5		0.3	12.7	(+)	(−)
43/F	(−)	152/95	2.8	Rt 3	32		<0.8	11.1	(−)	
55/F	(−)	180/90	2.7	Lt 4	53		0.2	15.1	(−)	(−)
64/M	(−)	132/80	2.7	Rt 1.7	32.4	(+)	<0.1	15.5	(−)	(−)
43/F	(+)	190/114	2.9	Rt 4.3	42.2		0.3	21.9	(−)	(−)
46/F	(−)	174/104	2.3	Rt 2.2	83.2		0.8	7.6	(+)	(+)
80/F	(−)	200/100	3.1	Rt 1.5	21.4		0.4	18.7	(−)	(−)
35/F	(−)	159/112	2.6	Lt 3	67.3	(+)	0.2	9.9	(−)	(−)

ACTH (pg/ml)	Urinary 17OHCS (mg/day)	DHEAS or DHEA	Adosterol scintigram	Post-surgical adrenal failure	References
	7		Lt	(+)	Komiya *et al*. 1979 (*10*)
		Low	Lt		Imai *et al*. 1991 (*11*)
		Low	Rt>Lt	(+)	Imai *et al*. 1991 (*11*)
10		Low		(+)	Allan *et al*. 2000 (*12*)
7	10.5		Lt		Honda *et al*. 2001 (*13*)
25	6.1	Low	Rt>Lt		Makino *et al*. 2001 (*14*)
<3		Low		(+)	Tanaka *et al*. 2002 (*15*)
27	5.2		Rt	(+)	Sugawara *et al*. 2003 (*16*)
9	10.4	Low	Rt		Fujii *et al*. 2005 (*17*)
5.3	8.5	Low	Lt		Present case

F, female; M, male; BP, blood pressure; Lt, left; Rt, right; Aldo, aldosterone; ACTH, adorenocorticotropic hormone; PRA, plasma renin activity; Dex, dexamethasone; 17OHCS, 17-hydroxysteroids; DHEA(S), dehydroepiandrosterone (sulfate).

825–844) and a house-keeping gene RPL19 (NM_000981: 401–420 and 571–590). For the quantification of CYP17, CYP11B2 and L19 mRNA levels, real-time polymerase chain reaction (PCR) was performed using a LightCycler-FastStart DNA master SYBR Green I system® (Roche Diagnostic Co., Tokyo, Japan) as reported in our previous study (*9*). As shown in Fig. 5, the ratio of CYP17/CYP11B2 in the present case (1.9) was situated between the levels of aldosterone adenomas (n=9; 0.9±0.1) and cortisol adenomas (n=5; 2.3±0.5). The ratio from a normal whole adrenal RNA was 2.8.

Discussion

In the present report, a rare case of primary aldosteronism complicated with preclinical Cushing's syndrome due to a single adrenocortical tumor was demonstrated. Increase in serum aldosterone levels and suppression of plasma renin release were diagnostic for primary aldosteronism. Although the serum cortisol levels were within the normal range, lowered secretion of ACTH and DHEAS, lack of dexamethasone suppression and a pattern of scintigraphy with suppression of the contra-lateral adrenal indicated autonomous cortisol secretion from the unilateral adrenal tumor.

Aldosterone-producing adenomas complicated with Cushing's syndrome or preclinical Cushing's syndrome have rarely been reported. As reviewed in Table 1, ten patients (9 females and 1 male) including the present case have been documented (*10–17*) since the first report by Komiya *et al*. (*10*). The mean age of these patients was 51.3 years and the mean diameter of the tumors was 2.7 cm. All the cases had marked hypertension with persistent hypokalemia (2.7 mEq/l on average). Overt features of Cushing's syndrome were observed in 1 case (*15*), in which the circulating cortisol level was relatively high (>20 μg/dl) and the plasma ACTH level was markedly suppressed. The other 9 cases exhibited normal serum cortisol levels in the morning. However, among these 9 patients, the cortisol secretion lacked a circadian rhythm in 6 cases and cortisol levels were not suppressed by 1-mg dexamethasone in 7 cases, suggesting preclinical/subclinical Cushing's state. Interestingly, 1 case showed a normal circadian pattern of cortisol secretion and normal suppression by dexamethasone (*16*). Nevertheless, postoperative adrenal insufficiency occurred in this case, possibly through suppres-

sion of the hypothalamo-pituitary-adrenal axis due to marginal but autonomous cortisol secretion (*16*).

In addition to decreased ACTH and increased midnight cortisol, lowered DHEAS levels are also useful for detecting subclinical Cushing's syndrome among patients with adrenal incidentaloma (*18*). DHEAS levels were lowered in 7 out of 10 cases, suggesting the presence of autonomous cortisol secretion. In the adrenal cortical scintigraphy, the lack of ^{131}I-adosterol accumulation in the contra-lateral side further indicated functional suppression of the contra-lateral adrenal, which was demonstrated in 6 out of 10 cases. These adrenal tumors were mainly composed of large clear cells mixed with scattered compact cells and accompanied by cortical atrophy in the adjacent tissues, although the adjacent changes were not observed in our case. The pathological difference may be reflected in varied levels of concurrent cortisol production in aldosteronomas. Immunohistochemical approaches demonstrated that such adenoma cells expressed P450c17 (*14–16*) or P45011β (*17*) in addition to P450aldo, suggesting the capability of cortisol production in these aldosterone adenomas.

In this regard, Adachi *et al*. reported that 6 cases who were initially diagnosed with primary aldosteronism (*19*) showed P450c17 expression in the adrenal tumors by immunohistochemistry, implying that concurrent secretion of aldosterone and cortisol may not be a rare condition. In our study, in order to clarify the expression levels of P450c17 compared with P450aldo, mRNA levels of CYP17 and CYP11B2 were determined in resected aldosterone- and cortisol-producing adenomas. As a result, the ratio of CYP17/CYP11B2 in the present case was clearly higher than that in aldosterone adenomas but lower than that in cortisol adenomas. These mRNA data and immunohistochemical findings of P450c17 clearly indicated that the present aldosterone adenoma was predominantly composed of clear cells with the ability to produce both cortisol and aldosterone.

According to the results from CRH loading tests, cortisol secretion by the contra-lateral adrenal was suppressed for approximately 3 months. Based on this finding, evaluation for the autonomy of cortisol secretion and contra-lateral adrenal function is clinically important to avoid the risk of adrenal failure after surgery for primary aldosteronism. Actually, when steroid replacement was discontinued, post-operative adrenal insufficiency was observed in 50% of the reported cases (Table 1). In very rare cases, co-existence of different adrenal tumors, each of which produces cortisol and aldosterone, has also been reported (*20*). It is therefore necessary to perform adrenal venous sampling when the bilateral adrenal lesions are suspected in such cases.

In summary, in cases of primary aldosteronism with low levels of basal ACTH and/or DHEAS, further endocrine examinations such as dexamethasone suppression, CRH test and adrenal cortical scintigraphy would be useful to determine the existence of autonomous secretion of cortisol. In such cases, preventive supplementation with hydrocortisone would be necessary to avoid a postoperative adrenal crisis.

Acknowledgements

We thank Professor Alan J. Conley of the Department of Population Health and Reproduction and the University of California, Davis, for kindly providing the rabbit antihuman P450c17 antibody.

References

1. Stowasser M, Gordon RD: Primary aldosteronism. *Best Pract Res Clin Endocrinol Metab* 2003; **17**: 591–605.
2. Otsuka F, Otsuka-Misunaga F, Koyama S, *et al*: Hormonal characteristics of primary aldosteronism due to unilateral adrenal hyperplasia. *J Endocrinol Invest* 1998; **21**: 531–536.
3. Miyoshi T, Otsuka F, Ogura T, *et al*: Aldosterone regulation for 18 years in a case of primary aldosteronism. *Am J Hypertens* 2004; **17**: 546–548.
4. Yokota K, Ogura T, Kishida M, *et al*: Japanese family with glucocorticoid-remediable aldosteronism diagnosed by long-polymerase chain reaction. *Hypertens Res* 2001; **24**: 589–594.
5. Hogan MJ, Schambelan M, Biglieri EG: Concurrent hypercortisolism and hypermineralocorticoidism. *Am J Med* 1977; **62**: 777–782.
6. Baert D, Nobels F, Van Crombrugge P: Combined Conn's and Cushing's syndrome: an unusual presentation of adrenal adenoma. *Acta Clin Belg* 1995; **50**: 310–313.
7. Hobma S, Hermus A, Pieters G, Smals A, Kloppenborg P: Concurrent hypercortisolism and hyperaldosteronism due to an adrenal adenoma. *Klin Wochenschr* 1990; **68**: 981–983.
8. Katabami T, Obi R, Shirai N, Naito S, Saito N: Discrepancies in results of low- and high-dose dexamethasone suppression tests for diagnosing preclinical Cushing's syndrome. *Endocr J* 2005; **52**: 463–469.
9. Inagaki K, Otsuka F, Suzuki J, *et al*: Involvement of bone morphogenetic protein-6 in differential regulation of aldosterone production by angiotensin II and potassium in human adrenocortical cells. *Endocrinology* 2006; **147**: 2681–2689.
10. Komiya I, Koizumi Y, Kobayashi R, *et al*: Concurrent hypersecretion of aldosterone and cortisol from the adrenal cortical adenoma. *Am J Med* 1979; **67**: 516–518.
11. Imai T, Seo H, Murata Y, Funahashi H, *et al*: Dexamethasone-nonsuppressible cortisol in two cases with aldosterone-producing adenoma. *J Clin Endocrinol Metab* 1991; **72**: 575–581.
12. Allan CA, Kaltsas G, Perry L, *et al*: Concurrent secretion of aldosterone and cortisol from an adrenal adenoma—value of MRI in diagnosis. *Clin Endocrinol* (*Oxf*) 2000; **53**: 749–753.
13. Honda T, Nakamura T, Saito Y, *et al*: Combined primary aldosteronism and preclinical Cushing's syndrome: an unusual case presentation of adrenal adenoma. *Hypertens Res* 2001; **24**: 723–726.
14. Makino S, Oda S, Saka T, *et al*: A case of aldosterone-producing adrenocortical adenoma associated with preclinical Cushing's syndrome and hypersecretion of parathyroid hormone. *Endocr J* 2001; **48**: 103–111.

15. Tanaka M, Izeki M, Miyazaki Y, *et al*: Combined primary aldosteronism and Cushing's syndrome due to a single adrenocortical adenoma complicated by Hashimoto's thyroiditis. *Intern Med* 2002; **41**: 967–971.
16. Sugawara A, Takeuchi K, Suzuki T, *et al*: A case of aldosterone-producing adrenocortical adenoma associated with a probable post-operative adrenal crisis: histopathological analyses of the adrenal gland. *Hypertens Res* 2003; **26**: 663–668.
17. Fujii H, Kamide K, Miyake O, *et al*: Primary aldosteronism combined with preclinical Cushing's syndrome in an elderly patient. *Circ J* 2005; **69**: 1425–1427.
18. Emral R, Uysal AR, Asik M, *et al*: Prevalence of subclinical Cushing's syndrome in 70 patients with adrenal incidentaloma: clinical, biochemical and surgical outcomes. *Endocr J* 2003; **50**: 399–408.
19. Adachi J, Hirai Y, Terui K, *et al*: A report of 7 cases of adrenal tumors secreting both cortisol and aldosterone. *Intern Med* 2003; **42**: 714–718.
20. Nagae A, Murakami E, Hiwada K, *et al*: Primary aldosteronism with cortisol overproduction from bilateral multiple adrenal adenomas. *Jpn J Med* 1991; **30**: 26–31.